

Lipid extracts isolated from heat processed food show a strong agglutinating activity against human red blood cells

I.S. Patrikios*

Department of C. togenetics, Institute of Neurolog and Genetics, C. prus

Received 1 March 2001; received in revised form 1 September 2001; accepted 2 October 2001

Abstract

In this study we investigated the possibility that the lipid components of foodstuffs and mass market oils undergo oxidative and thermal changes during storage, processing and cooking and so become agglutinins. The hemagglutinating activity of several mass market oils and several lipid mixtures isolated from different food items was evaluated against human red blood cells (RBCs) and against hamster RBCs. The unheated oils and the lipid extract of the unheated foodstuff had a very low agglutination titer but lysed red cells at high concentrations. When the same foodstuff items and oils were heated (100 °C for 24 h) in air, the isolated mixture of lipids as well as the oils show a strong hemagglutinating activity. Thin layer chromatography (TLC) of the lipid mixture, isolated from the processed foodstuff, as well as the heated samples of the oils showed appearance of high molecular weight molecules, possibly dimers and polymers. Light microscopy was used to characterize and visualize the agglutination process. Agglutination without lysis or fusion was observed. Agglutination may be the result of membrane properties alteration due to the oxidation product insertion or by hydrophobic side chain insertion into adjacent RBC membranes. We conclude that oils and foodstuff items when heated in air produce hemagglutinins against human RBCs with unknown and possibly toxic effects on human health. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Lipid hemagglutinins; Food processing; Thermal oxidation

1. Introduction

Lectins are specific proteins capable of binding to or agglutinating certain cells. Certain plant proteins, sometimes called phytohemagglutinins, can bind to and agglutinate red blood cells (RBCs). It has been found that they will bind to the surfaces of many other kinds of animal cells. They are especially abundant in plants, particularly of the legume family, and are also found in many invertebrate tissues.

The term “lectin” (Latine legere, “to pick or choose”) was first applied by Boyd and Shapleigh (1954) to seed extracts of plants, which could agglutinate and distinguish among human blood groups. Most remarkable and significant is the fact that some lectins preferentially agglutinate malignant tumor cells. Tumor cells must therefore have a different surface structure than normal cells. Materials which could clump RBCs were then called hemagglutinins. Hemagglutinins may be mem-

brane-bound or soluble in aqueous solution. Some lectins are known to agglutinate not only RBCs but also other kinds of cells such as lymphocytes, fibroblasts (connective-tissue precursors), spermatozoa, bacteria, and fungi (Sharon & Lis, 1972).

Lectins are known to cause toxic effects in people, but it is a matter of debate as to whether or not they can cause actual disease. Many lectins are resistant to cooking and digestive enzymes. They are present in much of our food. It is thus no surprise that they sometimes cause adverse reactions. In 1989, it was discovered that some food lectins pass through the gut wall and deposit themselves in distant organs (Freed, 1999; Wang, Yu, Campell, Milton, & Rhodes, 1998).

Stein and Cooper (1982) classified agglutinins into three categories: (1) antibodies, which are proteins of the immune system, are produced by lymphocytes or plasma cells, and may be elicited in response to antigens. They have a wide range of binding specificities, with two or more binding sites per molecule and they have been found only in vertebrates. (2) Lectins, which are glycoproteins or proteins. Their binding specificities

* Corresponding author. Tel.: +357-2-392694; fax: +357-2-392793.
E-mail address: ipatrik@mdrtc.cing.ac.cy (I.S. Patrikios).

are confined to carbohydrates and, as a general rule, they have two or more binding sites per molecule. They are present ubiquitously in viruses, bacteria, plants and animals. (3) Other receptor-specific substances are those agglutinins excluded from the strict definition of other two categories. These include the inducible, non-immunoglobulin heteroagglutinins of vertebrates and invertebrates and other lectin-like but possibly nonprotein (carbohydrate or lipid) agglutinins in invertebrates.

Stone (1946) observed that mixtures of pure lipids (phosphatidylethanolamine, phosphatidylcholine, sphingomyelin, cardiolipin, oleic acid (OA), stearic acid and glycol distearate) showed agglutinating properties similar to organic extracts of a wide variety of tissues. Tsivion and Sharon (1981) reported that polar lipid fraction from rat thymocytes, and certain bovine-brain lipids agglutinated some kinds of erythrocytes (RBC). Lai, So and Russell (1989) reported that fresh commercial dioleoylphosphatidylethanolamine (DOPE) did not have agglutination activity while samples which had been “aged” or been heated in water for 1 h or been heated dry for 3–6 h became powerful agglutinins. OA is one of the major components of biological membranes. In our hands, pure OA does not agglutinate red cells, but aged or heated samples agglutinate several species of red cells (Patrikios, Britton, Bing, & Russell, 1994).

There is a great deal of interest in the oxidation and thermal degradation of lipids. These changes can occur on ageing (Jurgens, Hoff, Chisolm, & Esterbauer, 1987). Oxidized lipids can alter the normal physiological activities of lipids, including regulation and membrane properties (Kagan, 1988). The fatty acid components of lipid foodstuffs undergo oxidative and thermal changes during storage, processing and cooking. These changes alter their nutritional properties. The biological effects that consumption of heated and/or oxidized fats may exert on human health have stimulated extensive research in recent years.

Digestibility was measured in experiments in which male Wistar rats were fed diets supplemented with unheated, heated, and 1:1 mixture of unheated/heated olive oils at 6, 12, and 20% (w/w) of diet. Fecal lipids were extracted after 14 days and analysed by a combination of adsorption and high-performance size exclusion chromatographies. Significantly lower digestibility values of thermal oxidized oils compared to fresh oils were found (Marquez-Ruiz, Perez-Camino, & Dobarganes, 1992). It was concluded that the excreted levels of altered compounds were two-fold higher for the heated-oil diet compared to the unheated/heated oil diet. Other experiments on compounds derived from thermally oxidized oils have reported digestibilities ranging from 30 to 70% (Bottino, 1962; Friedman, Horwitz, Shue, & Firestone, 1961). Complex mixtures of lipid degradation products occur in our daily diet, and further investigation is needed.

Polymerization of fatty acids, under oxidative or thermal treatments, are the subjects of intense interest in the synthetic chemical industries, especially as precursors for higher polymers.

The recent literature also suggests that fatty acids, particularly OA, may function as biological regulatory molecules but there are no reports of the effects of free fatty acid oligomers on biological materials.

Unsaturated fatty acids in food can easily produce free radicals after heating or exposure to light. Free radicals can drastically affect health. Free radicals serve normal functions, but can also injure, age, degenerate, and kill human cells and tissues. Many free radicals are produced every second and the body uses antioxidant (AO) nutrients like vitamins C, B3, and E, carotene, cysteine, selenium, bioflavonoids, and coenzyme Q10, as well as several enzymes containing zinc, manganese, and copper to neutralize them. During AO deficiency, free radical chain reactions can occur, leading to biologically inappropriate biochemical reactions, abnormal and toxic substances, and disease.

This study sought to determine: (1) if heating converts the lipid components of food to hemagglutinins against RBCs of human or other species; (2) to define the conditions under which oils and food lipid components are converted to hemagglutinins; and (3) to visualize and characterize the red cell–lipid interactions by microscopy.

This report describes results with mass market oils and a mixture of isolated food lipid extracts, which suggest that thermal oxidation converts them into species which interact with erythrocyte membranes in a different way to extracts of the unprocessed, and unheated precursors, i.e. cause hemagglutination.

2. Material and methods

2.1. Materials

HPLC grade solvents and fatty acids were obtained from Sigma (St. Louis, MO). The lipid standards were obtained from Larodan Fine Chemicals (Malmo, Sweden).

2.2. Lipid extraction

The effectiveness of the procedure will, to a large extent, depends on the chemical nature of the lipid components and the kind of complex or association in which they occur in the cell. There are three main types of associations in which lipids participate: (1) *van der Waals* or *hydrophobic* association in which neutral or non-polar lipids (sterol esters, glycerides, hydrocarbons and carotenoids) are bound through their hydrocarbon chains to other lipids and to hydrophobic regions of

proteins; (2) *hydrogen bonding, electrostatic and hydrophobic association* in which polar lipids (phosphatides, glycolipids, cholesterol) are bound to proteins by these forces, as in plasma membranes, mitochondria, endoplasmic reticulum and serum lipoprotein complexes; and (3) *covalent association* in which fatty acids, hydroxy acids or complex branched acids are linked covalently as esters, amides or glycosides to polysaccharide structures (in lipopolysaccharides of bacterial cell walls). The chemical nature of the lipids must also be taken into consideration in choosing an extraction procedure, especially to avoid oxidation/peroxidation of the double bonds.

For every 6 g of material 3 ml of water and 30 ml of methanol/chloroform (2:1, v/v) is added, and the mixture is blended for 2 min at room temperature. The homogenate is centrifuged for 5 min in a table-top International Clinical centrifuge, the supernatant is decanted, and the residue is re-extracted with 38 ml of methanol/chloroform/0.2 N HCl (2:1:0.8, v/v/v) by homogenization for 2 min, to ensure extraction of phosphoinositides. The combined supernatants are diluted with 20 ml each of chloroform and water and the phases separated by centrifugation. The lower phase (chloroform) is withdrawn, neutralized by dropwise addition of 0.2 N methanolic NH_4OH and concentrated in a rotary evaporator at 30–35 °C. About 10 ml of benzene is added to aid in removal of traces of water, and the residue is dissolved in chloroform/methanol (2:1, v/v) and stored at –10 °C until used.

2.3. Hemagglutination assay

Hamster blood was collected into 3.8% sodium citrate by cardiac puncture from live anaesthetized hamsters. Hamster and human RBCs were washed twice with 1 mM phosphate-buffered saline (0.85%)–0.01% sodium azide (PBS-N; pH 7.4). The volume of packed RBC was noted and diluted to 5% RBC (v/v) with PBS-N. PBS-N (100 μl) was placed in the control well No.1 of a microtiter plate and 50 μl into the wells numbered 2–12. Lipid preparations (50 μl , 0.3–0.5 mg/ml) were serially diluted in the wells numbered 2–12. These dilutions were done in duplicate. PBS-N (50 μl) was added to the serially diluted wells followed by 25 μl of 5% RBCs to all wells. The plates were shaken on a Tektator Shaker (Stuart Scientific, UK) for 3 min and incubated at room temperature. The plates were read at half-hour intervals by tilting them and observing the bottoms of the wells. The first well of each row served as control. Hemagglutination was recognized as the button-like settling of cells in contrast to flowing in the control wells. Titer is expressed as the highest dilution of test samples which still gives agglutination. Specific titer is defined as titer per mg lipid per ml.

2.4. Thin-layer chromatography

Silica gel G plates (20×20 cm; Macherey-Nagel, GmbH & Co., Germany) were prewashed in the developing solvent system: isooctane/isopropyl alcohol/acetic acid (95:5:1, v/v/v), air dried for 0.5 h and activated by heating for 1 h at 120 °C under vacuum (15 mm). From a stock solution of 0.5 mg/ml in chloroform/methanol (1:1v/v), 20–30 μg of sample was spotted onto silica gel plates using 2 μl Microcap pipettes (Blaubrand, Germany). Before development, the plates were dried using a hand-dryer on a cool setting for 5 min. The chromatography chamber, 26×7×24 cm (d×w×h), was saturated with vapor from the solvent system for 30 min before development of plates. The plates were allowed to develop until the solvent front was about 2 cm from the top, removed and air-dried for half an hour. The plates were visualized in iodine vapor.

2.5. Heated foodstuff preparations

Food samples of commercially prepared frozen pizza, fresh chopped pork meat, fresh chopped chicken meat, commercially prepared polybeef, hot dog, hamburger, tuna fish in olive oil, smoked bacon and chicken stock; corn oil, sunflower oil and olive oil were heated at 100 °C for 24 h in a metal heating block (USA/Scientific, Olala, Florida) in glass tubes open to air.

2.6. Preparation of lipid mixture dispersions

Samples dissolved well in ethanol, but high concentrations yielded separation of phases when mixed with PBS-N. Dispersions were prepared by diluting the samples to 0.5 mg/ml in ethanol. Dispersions of lipid extracts were filtered through LC PVDF Acrodisc membranes (Gelman) with size cut-off 0.2 μm , to remove particulate matter.

2.7. Light microscopy

Photographs were taken using a Cytovision Analyzer (Applied Image), attached to a Zeiss Axioskop microscope. Agglutinated cells were taken from microtiter plates containing samples which were being titrated and allowed to develop for approximately 30 min. The following preparations were used: (1) untreated human RBCs; (2) mixture of isolated lipid extract from different unheated food samples (1 mg/ml); (3) mixture of isolated lipid extract from different heated food samples (1 mg/ml); and (4) unheated and heated oil preparations (1 mg/ml). All samples were totally dissolved in pure ethanol. Fresh human and hamster RBCs were washed once with citrate because the cells retain their shape much better in citrate than in PBS, and therefore allow better photos to be taken. However, it should be noted

that cells washed in citrate will only maintain their integrity for approximately 2 h, and for that reason, only that portion of drawn blood needed for a single titer plate is washed.

3. Results and discussion

There are many studies in the literature about oxidation of lipids and fatty acids. But none have mentioned

Table 1

Hemagglutination, lysis (*L*) and specific activity of human red blood cells by lipid mixture extracts from heating mass market oils and foodstuff at 100 °C for 24 h^a

Food item ^b	Lysis		Titer ^c		Specific activity	
	U	H	U	H	U	H
Corn oil	<i>L</i> ³	<i>L</i> ²	2 ²	2 ⁸	2.2×10	5.6×10 ²
Sunflower oil	<i>L</i> ³	<i>L</i> ²	2 ²	2 ⁸	2.2×10	5.6×10 ²
Virgin olive oil	<i>L</i> ⁴	<i>L</i> ²	0	2 ⁸	0	5.6×10 ²
Poly beef	<i>L</i> ²	<i>L</i> ³	2 ³	2 ⁸	2.8×10	5.6×10 ²
Hot dog	<i>L</i> ³	<i>L</i> ³	2 ³	2 ⁸	2.8×10	3.2×10 ²
Tuna fish in olive oil	0	<i>L</i> ³	2 ⁵	2 ⁹	2.8×10	1.2×10 ³
Bacon	<i>L</i> ²	<i>L</i> ³	2 ⁴	2 ⁸	4.0×10	5.6×10 ²
Fresh chicken meat	<i>L</i> ³	<i>L</i> ³	2 ⁴	2 ⁶	4.0×10	1.5×10 ²
Fresh pork meat	<i>L</i> ³	<i>L</i> ³	2 ⁴	2 ⁶	4.0×10	1.5×10 ²
Chicken stock	0	<i>L</i> ³	2 ⁶	2 ⁸	1.5×10 ²	5.6×10 ²
Pizza	<i>L</i> ³	0	2 ³	2 ⁷	2.8×10	3.2×10 ²

^a Samples were completely dissolved in pure EtOH. The concentration was approximately 0.5 mg/ml. *L*^x is expressed as the highest dilution of test sample, which gives lysis. Titer is expressed as the highest dilution of test sample, which still gives agglutination. Specific titer is defined as titer per mg lipid per ml.

^b Food items heated (H) and unheated (U).

^c Titer remains the same after filtration through a 0.2-μm filter.

the hemagglutinating properties of the oxidation or thermal degradation products of the fatty acid components of lipid foodstuffs during storage, processing and cooking. These changes alter the nutritional properties of lipids.

The major causative factors in atherosclerosis are oxidized breakdown products of dietary fats (frying oils, precooked meat products, margarine and butter, and any food with substantial content of rancid fats; Navad, 1995; Schwartz, 1991). As they reach the bloodstream, oxidized food derivatives pose a direct threat to the vessel endothelia. McIntyre, Zimmerman, and Prescott (1999) reported that lipid oxidation is deleterious and some of the products of the attack are highly reactive species that modify proteins and DNA.

We became interested in studying the hemagglutinating activity of oils and food lipid extracts when we observed that OA samples exhibited hemagglutination activity after being kept frozen or refrigerated over a long period. The same result was obtained by heating a fresh sample in air. Since OA is found (free or as a lipid component) in most, if not all, oils and foods and thus in our everyday diet, it is possible that the same hemagglutinating activity would be found under the same conditions.

Mass market oils and foodstuffs that are commonly used in our everyday diet have been chosen to be the items under investigation. Heating oils and foodstuffs in air for 24 h at 100 °C produced strong hemagglutinins against human RBCs (Table 1). Specific activity, defined as titer per mg per ml, did not change with concentration of sample dispersions. Pure (fresh) samples of lipids and fatty acids do not have any titer against human RBC. Very low titer samples are the samples with titers 2² and 2³. Table 1 also showed that the chicken stock

Table 2

R_f values of the components of the heated mass market oils (24 h at 100 °C) and foodstuff items on silica gel TLC in isooctane/isopropyl alcohol/acetic acid (95:5:1, v/v/v)

Heated oleic acid ^a	<i>R_f</i> values of components ^b											
	1	2	3	4	5	6	7	8	9	10	11	12
						0.93				0.93		
	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
				0.48			0.48	0.54				0.55
Monomer	0.38				0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Dimer ^c	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Dimer ^d	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trimer	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
						0.07	0.07	0.07	0.07	0.07	0.07	0.07
						0.05	0.05	0.05	0.05	0.05	0.05	0.05
						0.00	0.00	0.00	0.00	0.00	0.00	0.00

^a The identification of the components of the fractions was by comparison with *R_f*s of the components of heated sample of oleic acid.

^b Components of the heated samples: 1, olive oil; 2, corn oil; 3, sunflower oil; 4, polybeef; 5, hot dog; 6, hamburger; 7, tuna fish in olive oil; 8, bacon; 9, chicken meat; 10, pork meat; 11, chicken stock; 12, pizza.

^c Linear dimer.

^d Cyclic dimer.

sample had a high titer before heating (2⁶) which increased even more after heating (2⁸). This was possibly due to the heating procedure used in the manufacturing process. Tuna fish in olive oil also showed high titer before heating (2⁵) and the highest when heated (2⁹). This may be because cold water fish oils contain unsaturated fatty acids which can be easily oxidized, as well as the olive oil present. The heating during the manufacturing process may also contribute to the high titer obtained. We also expect to see a strong hemagglutination activity with the unheated samples of the mass market oils, since commercial oil manufacture involves heating at high temperatures. However, this does not happen, possibly because the manufacturing pressing process at high temperatures takes only for few minutes and the oil is usually protected from exposure to air and light. Light produces free radicals in oils, and free radicals can produce changes in molecules that can

affect health. Byproducts, resulting from oils or food exposure to light, can be comparable to some byproducts resulting from the exposure of the same items to heating in air (free radicals, dimers, polymers, peroxides etc.). An average cooking temperature is 120 °C and the process takes place in air and light. These conditions are more than enough to convert all commercial oils used and the lipid content of cooked food to strong hemagglutinins with an unknown effect on human health. Oligomeric fatty acids are good agglutinins before heating (Patrikios et al., 1994). Table 1 showed that the poly-beef, hot dog and pizza samples had a very low hemagglutination activity (2³) before heating but the activity was very high after heating (2⁸). The hemagglutination activity of the bacon sample was also low before heating (2⁴) but increased after heating (2⁸). Fresh chicken meat and fresh pork meat samples both had low hemagglutination activity (2⁴) before heating which also increased

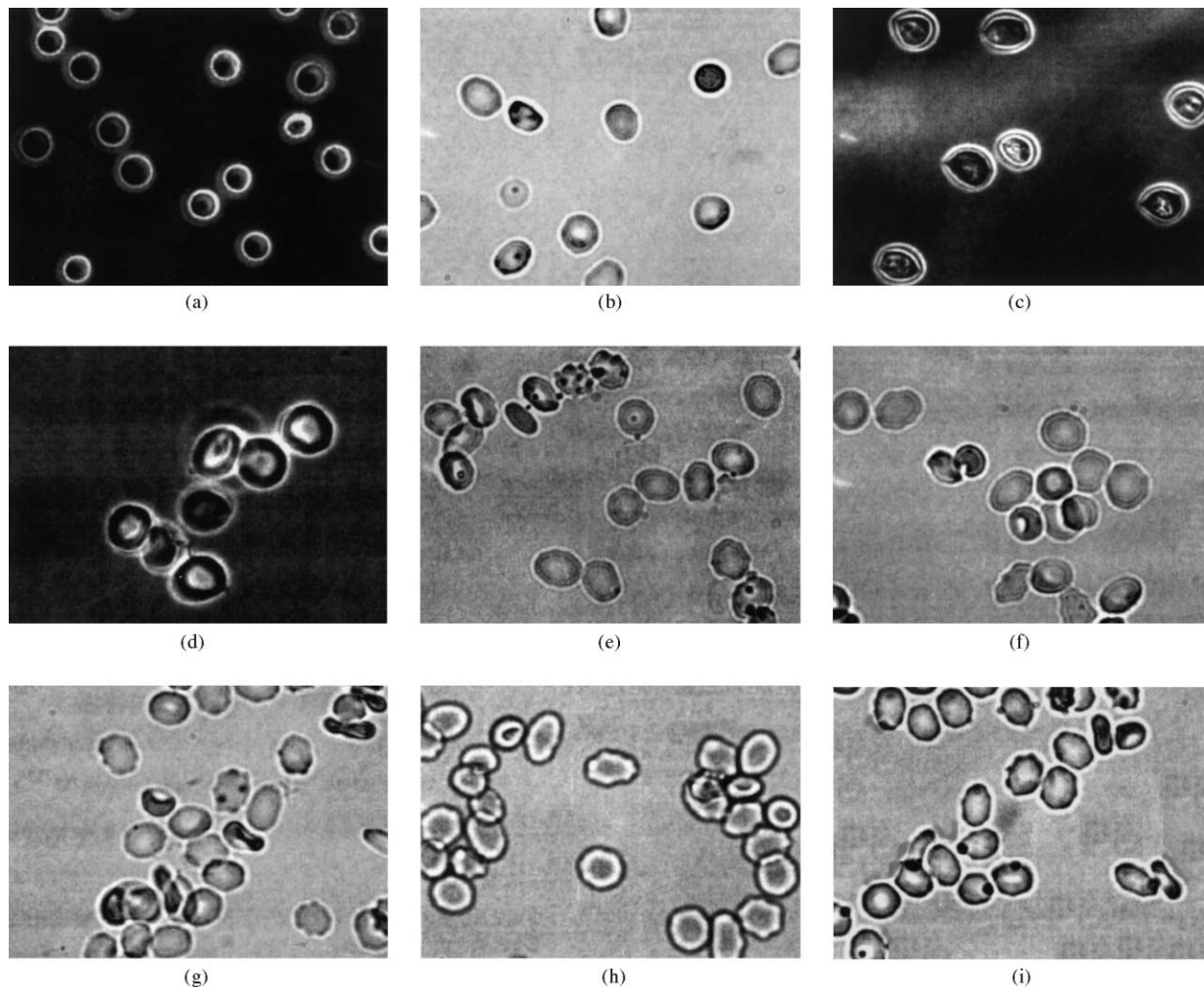


Fig. 1. Light micrographs of human red blood cells (RBCs) with the oils and the isolated mixtures of foodstuff lipids that show the highest specific activity when heated. (a) In citrate buffer with no additions, (b) in pure EtOH with no addition, (c) with olive oil (the figure has a RBC field distribution pattern representative for all of the other unheated oils tested; 1 mg/ml), (d) with heated (24 h at 100 °C) olive oil, (1 mg/ml) (e) with heated (24 h at 100 °C) poly-beef (1 mg/ml), (f) with heated (24 h at 100 °C) hot dog (1 mg/ml), (g) with heated (24 h at 100 °C) tuna in olive oil (1 mg/ml), (h) with heated (24 h at 100 °C) bacon (1 mg/ml), and (i) with heated (24 h at 100 °C) chicken stock (1 mg/ml).

after heating (26). Fresh chicken meat and fresh pork meat samples showed the lower increase of activity when heated; this might be because the fatty acids are mostly found within the cell membrane bilayers and cannot be easily oxidized.

The components of the heated oils and the lipid food extracts were resolved by thin layer chromatography on silica gel in a system that discriminates by the number of carboxyl groups (Table 2). The identification of the component of the fraction was carried out by comparison with R_f s of the components of a heated sample of OA. Some byproducts in the oil samples, as well as in the isolated lipid mixture extracts, showed the same R_f values as the corresponding R_f s of the monomeric, dimeric and polymeric byproducts of the heated sample of pure OA. This might be the result of hydrolysis, which can take place under these conditions (heating in air), of the dioleoyl phospholipids, releasing OA or any other unsaturated fatty acid from any phospholipid or triglyceride etc., resulting in dimerization and polymerization which have been shown to produce strong hemagglutinins. It is also possible that other lipid components from the isolated lipid extract, such as dioleoyl and dilinoleyl phospholipids or other unsaturated fatty acids, may be converted to agglutinins as well, or play a synergistic role. Since polymerization is apparently necessary and sufficient for the activity, this common denominator might be the reason for the hemagglutinating activity. Each isolated lipid extract showed a different RBC specificity profile, although they all showed high titers for human and hamster RBCs with hamster being higher. This may be due to the topographical differences and the different components present on the cell membranes between the two species.

Fig. 1 shows Human RBCs that were suspended in 3.8% citrate (control) and Human RBCs which were mixed with the isolated lipid extract preparations. The concentrations of the samples were identical. As seen in Fig. 1, the heated oils and preparations of the lipid extracts isolated from the processed foodstuff gave similar agglutination patterns. Mixtures of chains and rosettes were observed, but no fusion or lysis was apparent. Agglutination was found to be time-dependent and to involve cell clumping but not cell fusion. The agglutination, which we observed, may be due to a partial insertion of the oxidized lipid mixture extract or the oxidized oils into red cell membranes in a manner which links red cells rather than perturbing membrane permeability properties. However, another possibility is that the insertion of the oxidized lipid mixture into the red cell membrane facilitates local phase changes, which favor agglutination. We concluded that mass market oils and foodstuffs can undergo nutritional (especially lipid composition) damage when heated in air, with a direct effect on human health. Possibly, this can also happen when the oils and foodstuffs stand for long per-

iods of time on the shelf in direct sunlight, but this needs more extensive investigation.

Acknowledgements

The author wishes to thank the Cyprus Institute of Neurology and Genetics for hosting the project, and special thanks to Dr. Philippos Patsalis for his genuine offer of Lab-space and equipment. This work was supported by a grant from the Research Promotion Foundation of Cyprus RPF 06/99.

References

- Boyd, W. C., & Shapleigh, E. (1954). Specific precipitating activity of plant agglutinins (Lectins). *Science*, 119, 419.
- Bottino, N. R. (1962). Nutritive value of methyl linoleate and its thermal decomposition products. *Journal of American Oil Chemical Society*, 39, 25–31.
- Freed, D. L. J. (1999). Do dietary lectins cause disease? *British Medical Journal*, 318, 1023–1024.
- Friedman, L., Horwitz, W., Shue, G. M., & Firestone, D. (1961). Heated fats II. The nutritive properties of heated cottonseed oil and of heated cottonseed oil fractions obtained by distillation and urea adduct formation. *Journal of Nutrition*, 73, 85–89.
- Jurgens, G., Hoff, H. F., Chisolm, G. M., & Esterbauer, H. (1987). Modification of human serum low density lipoprotein by oxidation-characterization and pathophysiological implications. *Chemical Physiology of Lipids*, 45, 2–4.
- Kagan, V. (1988). *Lipid peroxidation in biomembranes*. Boca Raton: CRC Press.
- Lai, P.-S., So, L.-P., & Russell, C. S. (1989). A lipid-associated sulphated proteoglycan from *nereis* coelomic fluid is a hemagglutinin. *Comparative Biochemistry and Physiology*, 93B, 859–865.
- Marquez-Ruiz, G., Perez-Camino, M. C., & Dobarganes, M. C. (1992). Digestibility of fatty acid monomers, dimers and polymers in the rat. *Journal of American Oil Chemical Society*, 69, 930–934.
- McIntyre, T. M., Zimmerman, G. A., & Prescott, S. M. (1999). Biologically active oxidized phospholipids. *Journal of Biological Chemistry*, 274, 25189–25192.
- Navad, M. (1995). Pathogenesis of atherosclerosis. *American Journal of Cardiology*, 76, 18c–23c.
- Patrikios, S. I., Britton, O'N., Bing, D. K., & Russell, C. S. (1994). Heating unsaturated fatty acids in air produces hemagglutinins. *Biochimica et Biophysica Acta*, 1212, 225–234.
- Schwartz, C. J. (1991). The pathogenesis of atherosclerosis: an overview. *Clinical Cardiology*, 14, 1–16.
- Sharon, N., & Lis, H. (1972). Lectins: cell-agglutinating and sugar-specific proteins. *Science*, 177, 949–959.
- Stein, E. A., & Cooper, E. L. (1982). Agglutinins as receptor molecules: a phylogenetic approach. In E. L. Cooper, & M. A. B. Brazier (Eds.), *Developmental immunology: clinical problems and aging* (pp. 85–98). New York: Academic Press.
- Stone, J. D. (1946). Lipid hemagglutinins. *Australian Journal of Experimental Biology and Medical Science*, 24, 197–205.
- Tsivion, Y., & Sharon, N. (1981). Lipid-mediated hemagglutination and its relevance to lectin-mediated agglutination. *Biochimica et Biophysica Acta*, 642, 336–344.
- Wang, Q., Yu, L.-G., Campell, B. J., Milton, J., & Rhodes, J. M. (1998). Identification of intact peanut lectinin peripheral venous blood. *Lancet*, 352, 1831–1832.